Drug-induced liver injury

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Abstract: Drug-induced liver injury (DILI) is an adverse reaction to drugs or other xenobiotics that occurs either as a predictable event when the subject is exposed to toxic doses of some compounds (acetaminophen overdose) or in an unpredictable way with many drugs in common use. Drugs can be harmful to the liver in a susceptible subject on the background of genetic and environmental factors. This accounts for modifications in the hepatic metabolism and excretion of the agent leading to cellular stress, direct cell death, activation of an adaptive immune response and a failure to adapt with progression to overt liver injury. Idiosyncratic DILI is a relative rare liver disorder but can be severe and even fatal, presenting with a variety of phenotypes, which mimic almost every other liver disease. Diagnosis of DILI relies on the exclusion of other etiologies of liver disease as specific biomarkers are still lacking. Clinical scales such as CIOMS/RUCAM can support the diagnostic process but need a refinement. A number of clinical variables, validated in prospective cohorts, can be used to predict a more severe DILI outcome. Although no pharmacological therapy has yet been adequately tested in randomized clinical trials, corticosteroids can be useful, particularly in the emergent form of DILI related to immune checkpoint inhibitors.

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DRUG-INDUCED LIVER INJURY

Raul J Andrade [1,2], Naga Chalasani[3], Einar S. Björnsson[4], Ayako Suzuki [5,6], Gerd A. Kullak-Ublick[7,8], Paul B. Watkins[9], Harshad Devarbhavi[10] ,Michael Merz[7,11], M Isabel Lucena [12,2*], Neil Kaplowitz[13], Guruprasad P Aithal[14]*

Author addresses

[1]Unidad de Gestión Clínica de Enfermedades Digestivas, Instituto de Investigación Biomédica de Málaga-IBIMA, Hospital Universitario Virgen de la Victoria, Universidad de Málaga, Malaga, Spain.

[2]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain.

[3]Division of Gastroenterology, Indiana University School of Medicine, Indianapolis, Indiana, USA.

[4]Department of Gastroenterology, Landspitali University Hospital Reykjavik, University of Iceland, Reykjavík , Iceland and Faculty of Medicine, University of Iceland..

[5]Gastroenterology, Duke University, Durham, North Carolina, USA.

[6]Gastroenterology, Durham VA Medical Center, Durham, North Carolina, USA.

[7]Department of Clinical Pharmacology and Toxicology, University Hospital Zurich, University of Zurich, Zurich, Switzerland.

[8]Mechanistic Safety, Novartis Global Drug Development, Basel, Switzerland

[9]UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina; University of North Carolina Institute for Drug Safety Sciences, RTP, North Carolina, USA.

[10]Department of Gastroenterology and Hepatology, St. John's Medical College Hospital, Bangalore, India.

[11]Patient Safety, AstraZeneca, Gaithersburg, Maryland, USA

[12]Servicio de Farmacología Clínica, Instituto de Investigación Biomédica de Málaga-IBIMA, Hospital Universitario Virgen de la Victoria, UICEC SCReN, Universidad de Málaga, Málaga, Spain.

[13]Division of Gastroenterology and Liver Diseases, Department of Medicine, Keck School of Medicine, 2011 Zonal Avenue, HMR 101, Los Angeles, CA 90033, USA.

[14]Nottingham Digestive Diseases Centre and National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre at the Nottingham University Hospital NHS Trust and University of Nottingham, Nottingham, UK.

Correspondence

Raúl J Andrade
Email: andrade@uma.es

M Isabel Lucena
Email: lucena@uma.es

Author contributions

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Competing Interests

The authors declare no competing interests in this topic.

Abstract

Drug-induced liver injury (DILI) is an adverse reaction to drugs or other xenobiotics that occurs either as a predictable event when the subject is exposed to toxic doses of some compounds (acetaminophen overdose) or in an unpredictable way with many drugs in common use. Drugs can be harmful to the liver in a susceptible subject on the background of genetic and environmental factors. This accounts for modifications in the hepatic metabolism and excretion of the agent leading to cellular stress, direct cell death, activation of an adaptive immune response and a failure to adapt with progression to overt liver injury. Idiosyncratic DILI is a relative rare liver disorder but can be severe and even fatal, presenting with a variety of phenotypes, which mimic almost every other liver disease. Diagnosis of DILI relies on the exclusion of other etiologies of liver disease as specific biomarkers are still lacking. Clinical scales such as CIOMS/RUCAM can support the diagnostic process but need a refinement. A number of clinical variables, validated in prospective cohorts, can be used to predict a more severe DILI outcome. Although no pharmacological therapy has yet been adequately tested in randomized clinical trials, corticosteroids can be useful, particularly in the emergent form of DILI related to immune checkpoint inhibitors.

[H1] Introduction

Drug induced liver injury (DILI) is a term used to describe the unexpected harm that drugs in common use can cause to the liver, which include damage to hepatocytes and other liver cells. The main reason explaining the susceptibility of the liver to adverse drug reactions is probably its central role in biotransformation (metabolism) of xenobiotics entering the gastrointestinal tract.

Liver toxicity related to drugs has been classically divided into two varieties based on the presumed mechanism of action of the chemical compound: intrinsic and idiosyncratic. The intrinsic (or direct, predictable) type is dose-related and occurs shortly after exposure (hours to days) in the majority of individuals exposed to the drug (which is toxic at a dose threshold level)-. In contrast, the idiosyncratic (indirect, unpredictable) variety of DILI does not correlate with the dose and usually occurs in <1 of every 10.000-exposed individuals, and with a longer latency period (from a few days to several months)[1]. However, clinical observations during the last decades have somehow blurred the lines that distinguish these two types of hepatotoxicity. When not stated otherwise we use the term DILI for both intrinsic and idiosyncratic injury.

The main example of intrinsic DILI is acetaminophen (also known as paracetamol or APAP) hepatotoxicity, which accounts for ~50% of acute liver failure (ALF) cases in the US and some European countries[2,3]. Interestingly, a significant proportion of acetaminophen hepatotoxicity cases occurs unintentionally at doses slightly above the maximum recommended daily dose of 4 g, or even with repeated doses below this safety threshold[1,4]. Supposedly, a number of factors including fasting, alcoholism, concomitant use of other drugs and coexisting diseases, can decrease the toxic acetaminophen threshold dose by activating the generation of reactive drug metabolites via CYP2E1 and/or by depleting the hepatic glutathione concentration, which is the main detoxification pathway for acetaminophen toxic intermediates.

Idiosyncratic DILI, although not dose related, occur more frequently with doses of >50-100 mg/day[5]. Hence, a minimum dose, which probably varies among individuals, also seems to be necessary to trigger the cellular cascade of events leading to idiosyncratic liver damage. Importantly, idiosyncratic DILI can be severe and, in some cases, fatal. It accounted for 11% of ALF cases in the United States by 2013 [2], and represents a substantial concern for physicians, patients and drug companies. Indeed, idiosyncratic DILI remains a leading cause of terminating further drug development in investigational programs, and restrictions of use once the drug is on the market; 32% of drug withdrawals during the period 1975 to 2007 were attributed to hepatoxicity[6]. However, complete determination of the liver safety profile of a given drug requires considerable time after drug development, usually necessitating the exposure of hundreds of thousands patients to the compound.

Many drugs in common use have been associated with hepatotoxicity events[7], although the relative risk varies widely between drugs. Anti-tuberculosis therapy, in particular isoniazid, is the prototypical example of hepatotoxic drugs, causing overt liver injury in 0.1 to 1% of subjects[8]. On the other side of the spectrum are drugs such statins, which have been associated with hepatotoxicity in case reports and case series[9]. Considering the large number of individual exposed to these drugs, however, their hepatotoxic potential is very low, probably <1 in 50,000 treated patients[10].

The severity of DILI varies between patients, and depends on the drug type and several host factors. Some patients develop ALF and may require liver transplantation, whereas others can develop chronic DILI. In general, most patients make a full recovery. Although research over the last years has provided new data on DILI epidemiology and has enabled a better understanding

of its pathogenesis, significant gaps still remain particularly in the field of DILI prediction, diagnosis and therapy.

In this Primer we discuss the epidemiology, mechanisms, diagnosis, screening, prevention and management of DILI, including aspects of the quality of life and the outlook highlighting the areas for future research.

[H1] Epidemiology

Determining the true incidence of DILI worldwide is difficult given the diverse cultures, traditions, health care systems and lack of consistent reporting systems and definitions.

No studies have specifically analysed the trend in incidence of DILI over time. Two ongoing prospective studies in Spain and in the US have not demonstrated any major differences in the prevalence of DILI over time. These studies have though not been population based and have therefore not been able to analyse changes in the incidence of DILI over time.
However, in follow-up studies the proportion of herbal and dietary supplements (HDSs) out of all patients with DILI have been increasing in recent years [11,12]. Furthermore, increased use of biological agents such as infliximab has been associated with an increasing frequency of DILI among patients treated with these agents[13].

[H2] *Asia*

The only prospective nationwide study of DILI in Asia was undertaken in South Korea over a 2-year period in 17 referral university hospitals. The extrapolated incidence of hospitalization because of DILI in this study was 12 per 100,000 persons per year[14] of which traditional and herbal medicines were the most common cause, and were implicated in >72% of cases.[14] A recent retrospective study from China reported an estimated annual incidence in the general population of 23.80 per 100,000 persons much higher than that reported from western countries.[11] Indeed, traditional medicines are often integrated into the healthcare systems of technologically well advanced Asian countries, such as South Korea and Singapore[15]. In Japan, although traditional and herbal medicines are less integrated into its healthcare system, the incidence and proportion of DILI from traditional medicines is increasing[16]. The proportion of DILI from traditional medicines and dietary supplements vary substantially across Asia countries, with 15% in Japan[16], 26.8% in China[17] and 71% in Singapore[18] . In both China and India, the incidence of DILI caused by traditional medicines is increasing[19,20] .

In India and China, anti-tuberculosis drugs have been revealed as the most common and second most common causes of DILI through large case series[17,21] respectively. Indeed, in India, anti-tuberculosis DILI is a leading cause of ALF, which is not surprising given that India is home to 22.7% of the world's tuberculosis population[22], and given the hepatotoxic potential of 3 of the 4 first line anti-tuberculosis drugs (isoniazid, rifampicin and pyrazinamide).[23]

[H2] *Europe*

In a retrospective study of the General Practice Research Database (GPRD) in the United Kingdom, the annual incidence rate of non-fatal DILI was 2.4 cases per 100,000 persons[24]. In this study, 1,636,792 individuals registered in the GPRD database were followed for 5,404,705

person-years, and 128 patients were subsequently deemed to have developed clinically significant DILI as based on retrospective causality assessment of medical records [24]. In a retrospective analysis of 1,164 patients with liver disease seen at an outpatient hepatology clinic over a 10 year period in Sweden 6.6% of patients had at least possible DILI[25] . These data were extrapolated to show a calculated crude incidence of 2.3 per 100,000 individuals per year, mainly due to antibiotics[25]. In a population-based, prospective study of >81,000 individuals in France between 1997 and 2000, 34 patients had DILI, resulting in an annual crude incidence rate of ~14 cases per 100, 000 inhabitants[26]. By comparison, the annual incidence of DILI was 19 per 100,000 inhabitants in a more recent prospective, population-based study from Iceland [13]. Similar to other cohort studies from Europe[27,28], antibiotics were the most common drug class and amoxicillin-clavulanate was the most common single agent to cause DILI, occurring in 1 out 2,350 users of amoxicillin-clavulanate[13].

[H2] ***United States***

A recent study investigated the incidence of idiosyncratic DILI in the United States based on individuals presenting with suspected DILI to gastroenterologists in Delaware (which has an adult population of 934,948 individuals)[29]. Twenty individuals met the definition of DILI in 2014, and this yielded an annual incidence of 2.7 cases per 100,000 adults. In 14 individuals who were further characterized, 53% of cases of DILI were due to prescription medications (36% due to antibiotics) whereas 43% of cases were due to herbal and dietary supplements[29]. Another study investigated the population-representative incidence of drug induced ALF in Kaiser Permanente, an integrated healthcare system serving ~ 5.4 million individuals residing in Northern California[30]. While acetaminophen was the most common cause of drug induced ALF (56%), the incidence of ALF due to idiosyncratic DILI was 0.59 per 1,000,000 person-years. Herbal and dietary supplements were a more common cause of ALF than traditional prescription medicines in this study.

[H2] ***Other areas***

In 2011 a multinational prospective LatinAmerican DILI Network was setup bringing together hepatologists from 10 countries. This initiative follows the same structured protocol and adjudication criteria as the Spanish DILI Registry. Among the 330 well phenotyped DILI cases included, 60% with hepatocellular injury, amoxicillin clavulanate was the main implicated drug similarly to what is found in other prospective DILI registries. However, nitrofurantoin and cyproterone acetate distinctly stood out as culprit DILI drugs, reflecting the differences in pharmaceutical policies and patterns of drug use across countries[31] . In sub-Saharan Africa and other resource-limited regions traditional remedies represents the main source of pharmacological care but data on hepatotoxicity is scarce and mainly related to antituberculosis drugs in patients with human immunodeficiency virus infection[32].

Certain patient factors, such as older age, multiple drug use, and genetic variants,[13,24,33] have been shown to predispose DILI.

[H1] Mechanisms/pathophysiology

[H2] Normal drug metabolism and transport

The liver is an important target for drug toxicity because of its important role in removing drugs, especially lipophilic ones, from the circulation. The process of drug uptake into hepatocytes, their metabolism and elimination is controlled by large families of proteins whose individual expression and functions are under the control of genetic and environmental factors, including the effects of drug interactions and concomitant disease, all of which ultimately influence the accumulation (exposure) and lead to stress-promoting effects of drugs in the liver[34]. Drugs are taken up into hepatocytes passively, or by an array of transport proteins located in the basolateral plasma membrane (Figure 1), including members of the solute carrier family (SLCs), the organic anion transporting polypeptide superfamily (OATPs) [35], members of the organic anion transporter (OAT) family[36] and organic cation transporter (OCTs) family.

After uptake by hepatocytes, drugs are metabolized by phase I and phase II enzymatic reactions. Phase I metabolites usually have only minor structural differences from the parent drug but can exhibit very different pharmacological actions. Phase II metabolism involves conjugation of a drug or its phase I metabolite with endogenous molecules such as glucuronic acid, sulphate or glutathione; the product is more polar and does not usually exhibit pharmacological activity. Drugs and drug metabolites are effluxed from hepatocytes into bile or back into sinusoidal blood for subsequent renal excretion, mediated mainly by ATP-binding cassette (ABC) transporters such as the multidrug resistance gene product MDR1, also called P-glycoprotein (*ABCB1*; Figure 1) and anion exchange mechanisms.

[H2] Hepatotoxic substrates and metabolism

Human hepatocytes express the transporters OATP1B1 (encoded by *SLCO1B1*), OATP1B3 (encoded by *SLCO1B3*) and OATP2B1 (encoded by *SLCO2B1*[37]. Potentially hepatotoxic substrates include statins (used to treat hypercholesterolemia), and plasma statin levels - a risk factor for statin-induced myopathy - increase in the presence of OATP1B1 inhibitors such as cyclosporin A (an immunosuppressant) or gemfibrozil (a lipid-lowering agent)[38,39]. Several tyrosine kinase inhibitors (TKIs, which are small molecules used to treat various forms of cancer) are substrates and/or inhibitors of OATPs. Pazopanib has a boxed warning for hepatotoxicity in the US FDA label, but the mechanism of hepatotoxicity is not related to inhibition of OATP1B1[40]; pazopanib uptake is mediated by the organic cation transporter 1 (OCT1, encoded by *SLC22A1*)[41]. The FDA provides further guidance on in vitro metabolism-mediated and transporter-mediated drug-drug interaction studies with investigational drugs[42].

A known mechanism of DILI is the formation of reactive metabolites in phase I and II reactions[43]. The covalent binding of reactive metabolites to cellular proteins can lead to alteration of function or location of the target protein, or to the formation of immunogenic haptens, which can trigger a downstream immune response[44]. For example, the NSAID diclofenac can cause severe hepatotoxicity and has been shown to form reactive quinone imines through metabolism by CYP2C9 and CYP3A4 and acyl glucuronides through metabolism by UDP-glucuronyl transferase (UGT) 2B7 [45]. Lumiracoxib and troglitazone, both of which caused fatal hepatotoxicity leading to market withdrawal, form reactive quinine metabolites[46,47]. To estimate the clinical risk of hepatotoxicity *in vitro*, the bioactivation potential is determined by glutathione trapping assays, mechanism-based CYP inactivation screens or covalent-binding assessment using radiolabeled compounds[48]. The detection of stable detoxification products

such as glutathione adducts or dihydrodiols in the metabolic pattern can indicate metabolic activation, as can time-dependent inhibition of an enzyme, which predicts the formation of reactive metabolites in >90% of cases. Reactive metabolites formed by CYP2C9, CYP1A2 and other selected enzymes have a higher likelihood of being associated with clinical observations[49].

A possible mechanism of DILI is inhibition of the bile salt export pump BSEP (*ABCB11*)[50], which increases intracellular concentrations of bile salts. Bile salts damage mitochondria[51], leading to cytotoxicity and liver injury[52]. Potent BSEP inhibitors include bosentan (used to treat pulmonary hypertension and has a boxed warning for hepatotoxicity) and cyclosporine A, which can lead to drug-induced cholestasis in clinical routine[53–55]. The major metabolite of the antidiabetic hepatotoxic drug troglitazone, troglitazone sulfate, competitively inhibits BSEP and accumulates in hepatocytes, thereby leading to an increase in intracellular bile salt concentrations and consequently mitochondrial damage[56]. As conjugated anionic drug metabolites are substrates of MRP2 (*ABCC2*)[57], genetic variants of this transporter have been associated with DILI[58–60]. Genetic variants of *ABCG2* (which codes for the breast cancer resistance protein BCRP) have been associated with hepatotoxicity induced by the TKI sunitinib[61].

Dysfunction of the multidrug resistance gene product 3 (MDR3, encoded by *ABCB4*), which translocates phosphatidylcholine from the inner to the outer leaflet of the lipid bilayer, is associated with various forms of cholestasis[62]. Phospholipids are an essential lipid component of bile that solubilize cholesterol in phospholipid-cholesterol vesicles. In addition, it is thought that the phospholipids protect the cholangiocytes from bile acids by keeping them in micelles, and that it is “naked” bile acids that damage cholangiocytes and cause cholestatic or mixed injuries. MDR3 is inhibited by certain drugs such as the antifungal agent itraconazole, resulting in reduced phospholipid output into bile[63]. Damage to cholangiocytes and small bile ducts can impair bile flow, leading to hepatocellular retention of cholephilic compounds and thereby to cholestatic liver injury. Antifungal azoles also inhibit BSEP and the combined inhibition of MDR3 and BSEP represents a dual mechanism by which azoles cause DILI in susceptible patients.

[H2] ***Cell death, adaptation and progression of injury***

Intrinsic DILI generally refers to direct toxic stress leading to cell death of hepatocytes (sometimes sinusoidal endothelial cells are the principal target) mediated by a reactive metabolite or a parent drug interfering with specific cell functions. This is mediated by increased oxidative or redox stress, mitochondria dysfunction, endoplasmic reticulum (ER) stress, or DNA damage [1,34,64,65]. As these progress unchecked, cell death occurs (Figure 2). Innate immune responses including activation of resident liver Kupffer cells and NK/NT cells as well as various cytokines, chemokines such as TNF, IL-1B, IL-8, IL-6, CXCL10, and infiltrating leukocytes may amplify the cell death through death receptor signalling and inflammation[1,64].

The lethal outcomes in hepatocytes in DILI are considered to be a result of mainly regulated modes of cell death, predominantly necrosis and apoptosis. A final pathway leads to complete collapse of mitochondria by increased permeability of the inner and outer membrane resulting in downstream consequences. The mitochondria membrane transition pore (MMTP) complex becomes dysregulated by the contribution of stress signal transduction (MAPK) which amplifies direct effects of toxic metabolite in mitochondria (e.g. acetaminophen toxicity)[66]. Alternatively, the intrinsic stress can activate inhibitor caspases (e.g. caspase 8) and Bcl family (e.g. Bid, Bax) of proteins which selectively permeabilize the outer mitochondrial membrane, releasing

cytochrome c which activates the executioner caspases (e.g. capase 3,7)[67]. Furthermore, the release of damage associated molecular patterns (DAMPS) from hepatocytes which may activate innate immune responses leading to death receptor induced apoptosis (TNF-R, FAS, TRAIL). Aside from acetaminophen toxicity, the relative contribution of necrosis versus apoptosis is largely undetermined with other intrinsic DILI toxins. Alternative mechanisms of regulated necrosis have emerged in recent years: Necroptosis, pyroptosis, ferroptosis. It is unknown if any of these are relevant to acute or chronic DILI, although perhaps they are important in NASH or ASH and autoimmune hepatitis[68]. In acute DILI, the weight of evidence indicates that necroptosis (RIPK3/ MLKL dependent cell death) plays no or minimal role, likely because RIPK3 is not expressed under basal conditions[66].

In contrast to intrinsic DILI, idiosyncratic DILI occurs in a small proportion of patients exposed to a drug, reflecting the important contribution of the host mediated by genetic and environmental factors. Currently, the preponderance of evidence is that idiosyncratic DILI is usually dependent on the adaptive immune response of the individual, determined by HLA polymorphisms and other contributing factors of immune activation, targeting neoantigen (hapten peptide) presented by one or more specific HLA alleles[69,70]. However, although unique HLA types appear to be important determinants of the immune response to reactive metabolites or parent drugs in some cases, most of the population with a specific drug-related risk HLA haplotype are unaffected by the exposure to drug, suggesting that other factors are involved. The identity of the other factors are not well defined. However, the extent of underlying drug-related toxic stress may be upstream (co-activator) of the development of an adaptive immune response.

As previously mentioned, idiosyncratic DILI has its onset after a variable but sometimes long latency (most <6 months) and is not dose-related but mainly occurs with dose of drugs above 50-100mg/day[71]. This probably reflects the fact that there is a threshold for activation of the immune system. Clearly lipophilicity of the drug, high daily dose, and its metabolism in the liver are key factors in achieving sufficient toxic exposure of hepatocytes to drugs, a prerequisite for most DILI cases[72]. An emerging area of interest is the microbiome which may affect the hepatic responses to toxic drugs[73]. The adaptive immune system plays a major role in the pathogenesis of idiosyncratic DILI. The adaptive immune system can be activated by covalently binding drugs (hapten hypothesis) leading to HLA restricted presentation of a peptide adduct by the immune system. In rare cases a drug may directly bind to certain HLA molecules or TCR and activate an immune response (Figure 1). Alternatively, in some instances a drug or metabolite may alter the HLA binding groove leading to misdirected peptide presentation.

A potential unifying aspect of both intrinsic and idiosyncratic DILI has been demonstrated using in vitro systems[74]. Using these test systems to identify toxic stress in the absence of innate or adaptive immune system may suggest that hepatocyte stress promotes neoantigen formation upstream of immunity and/or generates signals that co-activate the immune response. Aside from the contributions of drug-induced mitochondrial dysfunction, oxidative stress, proteostatic ER stress, all of which are interrelated, a variety of evidence suggests that drugs which inhibit BSEP potentially are more likely to cause idiosyncratic DILI. This has led to the hypothesis that the bile acid retention in hepatocytes can induce stress mediated by all the cellular and biochemical contributors listed above. Furthermore, bile acids can induce hepatocyte apoptosis

through increased plasma membrane targeting of death receptors, enhancing ligand independent activation leading to apoptosis or sensitizing to ligand (TNF, FASL, TRAIL) dependent apoptosis due to increase plasma membrane content of death receptors[75].

One important modulating factor in idiosyncratic DILI is the interplay between the onset of the immune activation and the participation of immune tolerance. Several examples of the importance of immune tolerance have been demonstrated in recent mouse studies in which the inhibition of several of the key players in immune tolerance have unmasked liver injury, as well as actually worsening DILI from its onset[76,77]. The proof of this mechanism in humans is lacking but it likely exists. It is clear that drugs, which are used to break immune tolerance in treatment of cancer, can lead to an autoimmune-like acute injury to the liver by eliminating the immune privilege, which is characteristic of the liver. Thus, one could speculate that the near universal stress in the liver due to parent or metabolized drugs, given at or above the dose threshold, may begin to cause liver injury, which either is below detection or associated with mild ALT elevations that disappear with continued exposure to a drug. Thus, adaptive responses, which dampen the initial toxic stress or by the development of immune tolerance may inhibit the progression to overt liver injury. In theory, this adaptation may begin before any sign of liver injury appears (e.g. ALT increase) or after the initial immune mediated liver injury is detected (delayed asymptomatic ALT increases that resolve despite continued treatment with the offending drug), referred to as clinical adaption. Accordingly, overt liver injury may be a failure of immune tolerance[76,77]. Though somewhat speculative, this hypothesis is plausible and provides a framework for future studies.

[H2] Interplay between drugs and host factors

Specific drug properties, such as high daily-recommended dose, high lipophilicity, BSEP inhibition, reactive metabolite formation, mitochondrial toxicity and induction of oxidative stress, have been associated with drugs that possess hepatotoxic potential in humans.[78] In addition, patient factors can predispose to DILI (see Epidemiology, above). However, each of the elements alone does not accurately dictate the risk of DILI in humans, corroborating with a multifactorial nature of this disease. As detailed in the above sections, mechanisms involved in DILI are multiphasic: early phases (up to the initiation of cellular damage) are more drug-specific and are primarily influenced by drug exposure (e.g., dose, duration) and certain drug properties. In contrast, later phases are not specific to drugs and are defined by how the host responds to toxic stress and induces orchestrated cellular adaptation, immune responses, and tissue repair processes. Drugs and host factors influence multiple mechanisms and thus likely interact at different levels, defining DILI risks, clinical phenotypes, and outcomes in a sophisticated manner[78] (Table 1).

Evidence of drug-host interplay in DILI is rather scarce. In vitro studies, primary hepatocytes derived from men and women respond differently to various toxic compounds, suggesting drug-sex interplay at a cellular level[79]. In vivo animal studies, sex differences in susceptibility to DILI depends on models: male dominance in liver injury induced by acetaminophen[80–82] and cocaine (only after the onset of puberty)[83,84] and female dominance in halothane[85–88] and in another immune-mediated DILI model[89]. In humans, age, sex, and a proxy of women's menopausal status (i.e., 50 years) significantly influence drug-specific reporting frequencies of liver events in the

World Health Organization VigiBase™[90,91], and influence clinical and histologic phenotypes of DILI[92,93]. Drugs associated with sex-/age-biased reporting frequencies of liver events showed distinct properties[90]. For example, drug properties such as mitochondrial toxicity, reactive metabolite formation, and BSEP inhibition are more prevalent among drugs with women-biased reporting frequencies[90]. Drug properties of high lipophilicity, biliary excretion, higher transporter inhibitions, C_{max} (the maximum serum concentration that a drug achieves) and plasma protein binding, yet shorter plasma elimination are more prevalent among drugs with old age-biased reporting frequencies[90,91]. In addition, drug properties, host factors, and their specific interactions can influence a likelihood of delayed onset of DILI[94].

Despite the scarcity of the data, emerging evidence suggests the significance of considering both drug and host together in assessing DILI risks. Future methodological implementation to cope with the complexity in DILI mechanisms and new human data sources that provide sufficient size and statistical power to address drug-specific DILI risk factors and drug-host interplay (e.g., big data analysis) are needed.

[H1] Diagnosis, screening and prevention

[H2] *Clinical phenotypes and case characterization*

The clinical manifestations of DILI are heterogeneous. Indeed, DILI can mimic acute and chronic liver diseases of varied aetiology, and symptoms can include fever, nausea, vomiting, jaundice, dark urine, right upper quadrant pain and itching. Certain drugs have signature injury patterns (e.g., acetaminophen, amiodarone, diclofenac and isoniazid for hepatocellular injury; anabolic steroid, captopril, and erythromycin for cholestatic injury) but others, such as atorvastatin, allopurinol, amoxicillin-clavulanate, show various DILI manifestations (Table 2)[95]. In addition, adverse reactions from a single drug can present with different phenotypes in different individuals, varying from asymptomatic liver biochemical test abnormalities to acute and subacute hepatic liver failure.

Although genome wide association studies in DILI performed during the past decade indicate that adaptive immunity plays a major role in disease pathogenesis[96], a majority of DILI episodes do not demonstrate immunological features. Clinical features of immune mediated or hypersensitivity drug reactions are seen in a quarter of patients and include fever, cutaneous rash, facial periorbital oedema, lymphadenopathy, eosinophilia, lymphocytosis or presence of reactive lymphocytes, or arthralgia[97]. For example, antiepileptic agents carbamazepine and phenytoin-induced liver injury are most commonly associated with cutaneous hypersensitivity features[98] and dapsone-induced liver injury is associated with cutaneous hypersensitivity features in 90% of patients[99]. The skin rashes may vary from non-specific morbiliform rashes to severe lesions such as erythema multiforme, DRESS (drug reaction with eosinophilia and systemic symptoms) syndrome or Stevens-Johnson syndrome/ Toxic epidermal necrolysis (SJS/TEN)[100,101].

Some other drugs such as alpha-methyl dopa, nitrofurantoin and minocycline are associated with features indistinguishable from autoimmune hepatitis with presence of anti-nuclear antibodies, hypergammaglobulinemia and liver biopsy features compatible with autoimmune

hepatitis[102]. Autoimmune like hepatitis associated with nitrofurantoin and minocycline are characterized by a prolonged latency to detection of more than a year[11].

[H3] A step-wise approach to clinical diagnosis.

The majority of DILI cases are suspected in any individuals with increased aminotransferase (AST/ALT) and/or alkaline phosphatase (ALP) levels beyond a certain threshold sometimes accompanied by raised total bilirubin (TBIL) values (Box 1) detected in the course of an investigation for non-specific symptoms or during a diagnostic-workup in patients who present with an acute viral hepatitis-like syndrome (Figure 3), the latter usually not pointing to drug etiology[95], unless there are associated skin or other systemic features that can reinforce the suspicion of drug toxicity[103]. Notably, the level of elevation of liver enzymes alone is not sufficient to reflect the severity of DILI[104,105]; the development of ascites, coagulopathy and/or encephalopathy indicates severe disease[104]. Asymptomatic elevations of transaminases that occur following exposure to medications and may either resolve with continuation of drugs or following decrease in dose are characteristic of anti-tuberculosis drug or statin therapy[106]. On the basis of results from liver biochemical tests, in most instances patients with suspected DILI are classified as hepatocellular, cholestatic or mixed DILI (Table 2).

A first prerequisite for DILI diagnosis is a high degree of suspicion and consequently a careful inquiry on prescription medication and over the counter drugs (acetaminophen) exposure, recording start and stop dates, as well as the exposure to herbal and dietary supplements (often overlooked by the physician)[49]. Information on latency, course of reaction upon pharmacological therapy discontinuation, and time to resolution is needed to establish a compatible temporal relationship with the suspected causative agent. Time to onset varies considerably; most patients experience DILI within the first 3 month of therapy, although in some instances (e.g. amoxicillin-clavulanate related DILI) symptoms can present with a considerable delay after treatment interruption[49].

The diagnosis of DILI currently relies on the exclusion of alternative causes (TABLE 3). This encompasses a medical history to exclude alcohol abuse, sepsis, congestive heart failure, a search for recent episodes of syncope or hypotension (which would indicate ischemic hepatitis), comorbidities, the assessment of the subject's risk behavior for acquisition of viral hepatitis, as well as the local burden of infectious diseases that might involve the liver[49]. The pattern of injury provides guidance in the additional investigations required. For example, a cholestatic anicteric pattern requires the exclusion of primary biliary cholangitis and primary sclerosing cholangitis, whereas in jaundiced patients a search for benign/malignant obstruction of the biliary tract should be prompted. Indeed, liver imaging is routinely used in the evaluation of patients with liver injury and all patients with suspected DILI should undergo abdominal ultrasonography to exclude biliary obstruction and focal lesions. In those with a cholestatic type of liver injury or in those with associated abdominal pain, additional imaging, such as magnetic resonance colangiography or computerized tomography might be required despite normal abdominal ultrasound.

Screening for viral hepatitis A (IgM-Anti HAV) B (IgM-AntiHBc, HBsAg) and C (anti-HVC) is mandatory in individuals with suspected DILI, expect for those with a pure cholestatic pattern.

In addition, assessing for RNA-HCV, which has been found to be present in 1.3% of cases initially believed to be DILI[107]is also required. In HBsAg carriers, hepatitis B virus DNA should also be tested to exclude chronic hepatitis B virus reactivation. Hepatitis E is an emergent disease in Western countries and is increasingly diagnosed in patients being evaluated for the inclusion in DILI Registries where anti-HEV IgM seroprevalence has ranged from 3% to 8% [108,109]. Accordingly, HEV should be tested for in all patients with suspected DILI through detection of HEV RNA, and anti-HEV IgM/IgG antibodies. Patients with hepatocellular pattern of injury should be also worked up for the hallmarks of autoimmune hepatitis (AIH) including anti-nuclear autoantibodies (ANA) and anti-smooth muscle autoantibodies (ASMA) and serum IgG. Nevertheless, a phenotype of AIH with its typical laboratory features is a characteristic signature of several drugs including nitrofurantoin, minocycline, anti-TNF-α and statins, which makes the differential diagnosis between this particular phenotype of DILI and classical AIH a challenge[49]. Indeed, a liver biopsy, which is not generally required for evaluation of a patient with suspected DILI, is justified when autoimmune features are present as it may provide important diagnostic clues; for instance in a small study, hepatocellular cholestasis and portal neutrophils was indicative of DILI, whereas the presence of fibrosis suggested AIH[110]. In another study using immunochemistry staining of liver biopsies portal infiltrates in DILI were formed predominantly by cytotoxic (CD8+) T cells, while in AIH there were prominent mature B cells (CD20+) [111].

In addition to use for detecting AIH, incomplete dechallenge upon drug discontinuation raises the possibility of an alternate aetiology or an atypical DILI phenotype (i.e. veno-oclusive disease) and liver biopsy can assist in this setting. Biopsy findings can also have prognostic value; a systematic review of liver biopsies from 249 patients with DILI from a prospective observational cohort showed that higher degrees of necrosis, fibrosis stage, microvesicular steatosis, and ductular reaction were indicative of a poorer prognosis, whereas eosinophils and granulomas were found more often in those with milder degree of DILI[112]. Likewise, pathological assessment of DILI cases mainly presenting with a cholestatic pattern identified that bile duct loss was predictive of the development of vanishing bile duct syndrome causing progressive cholestasis leading to liver failure requiring transplantation or death[113].

Serial aminotransferases measurement until complete normalization is also crucial for diagnostic reassurance in DILI. A steady decline of aminotransferases upon drug discontinuation (dechallenge) supports the diagnosis, whereas worsening, persistence or incomplete resolution of laboratory abnormalities suggest competing etiology[49]. Nevertheless, clinicians should bear in mind that a fraction of DILI cases can evolve to acute liver failure or become chronic despite stopping the drug, which further challenges the diagnosis. Besides this, in few instances and upon careful questioning the patient might recall similar symptoms after a prior exposure to the agent and inadvertent drug rechallenge can be identified[114]. Overall, clinical symptoms can be informative to identify drug signatures, establish alternative causes and predict outcome.

[H2] ***Causality assessment tools***

A number of clinical scales to quantify the strength of association - the proof of causality, which is the Achilles heel of adverse drugs reactions - have been proposed in DILI. Indeed, a valid structured and objective approach for adjudicating DILI cases is needed for research purposes

and to add consistency to clinical judgment by providing a framework that systematize the features to be addressed in cases of suspected hepatotoxicity[115].

The general Naranjo Adverse Drug Reactions Probability Scale is a simple and easy to apply scale, based on ten "yes", "no" or "unknown or inapplicable" questions related to common evaluating criteria. However, it has demonstrated low sensitivity and reproducibility in a registry study due to the presence of confusing and not relevant questions to idiosyncratic DILI and therefore is not recommended for use in DILI[116]. Currently, the CIOMS/RUCAM scale is the only validated liver specific scale used by regulators, pharmaceutical industry and clinicians and has been recommended by experts for causality assessment in DILI[104,117–119].

The CIOMS/RUCAM scale is composed of seven criteria: a temporal association between drug exposure and DILI recognition, dechallenge (rate of improvement with drug cessation), risk factors for DILI, exclusion of all other relevant causes of liver disorders, known drug hepatotoxic potential, recurrence of liver injury on drug re-exposure and the potential influence of associated medications. This scale categorises DILI as definite or highly probable, probable, possible, unlikely and excluded. Once a clinician is convinced that the case may be drug-related applying the CIOMS/RUCAM scale can further standardize and support the assessment. However, blind application of this scale is not a proof of causality and may lead to biased conclusions, particularly in poorly documented cases. Indeed, the CIOMS/RUCAM scale is mainly for supporting rather than excluding causality in DILI and does not substitute "clinical judgment".

However, the CIOMS/RUCAM scale is complex, includes ambiguous definitions, lacks data to support the selection and weighting of component domains, has a strong dependence on rechallenge data[115] and cannot obtain high categories of probability in some cases as dechallenge data are not included[118]. Patients with underlying liver disease can obtain lower scores owing to liver test fluctuations[11]. These shortcomings can explain the inter observer variability and inconsistent test-retest reliability, even when this scale is used by expert raters [120]. Besides, the use of herbal and dietary supplements also complicates causality assessment, as there may be inaccuracies in the identification of the ingredients, pharmaceutical adulterants, chemical/botanical contaminations, lack of information on dose and duration of product consumption and the potential for use of various complex formulations of plants or extracts. Differences in herbal terminology and limited product label information, if any, further contribute to the complexities to assign causality in this context [12,121].

The US DILIN group uses a structured expert consensus opinion-based approach that has shown higher agreement rates and likelihood scores than CIOMS/RUCAM in assessing causality, although the inter-observer variability was high with both instruments[122]. The scoring criteria categorise DILI likelihood as definite (>95% likelihood), very likely (75–95% likelihood), probable (50–74% likelihood), possible (25–49% likelihood), and unlikely (<25% likelihood)[122,123]. It is understandable, as the authors acknowledge, that lack of reproducibility may be due to the absence of numerical scores for each of the items evaluated. Opinions between evaluators are very dependent on prior knowledge of the examiner or information provided. In addition, expert opinion can weigh into the assessment clinical "signatures" for DILI that are known to be characteristic for specific drugs. Nonetheless, its reliability in daily clinical practice[124].

Another important limitation of the CIOMS/RUCAM scale is that cannot discriminate between concomitant hepatotoxic drugs with the same temporal sequence. Probably in an attempt to circumvent this limitation, the liver specific Digestive Disease Week-Japan (DDW-J) scale, modified from the CIOMS/RUCAM scale, includes an in vitro drug lymphocyte transformation test (LTT, which assesses whether the DILI reaction is mediated by a T-cell response against the drug), subject with an apparent hypersensitivity reaction has become sensitized to a specific drug the reactions were mediated by an allergic mechanism) in its evaluation criteria[125]. The lack of standardization among laboratories has prevented its generalization. Indeed, a modified LTT measuring granzyme B and cytokine production was neither reliable for establishing causality[126]. In a further attempt to improve diagnostic capabilities, a hepatotoxicity assay using monocyte-derived hepatocyte –like cells from patients with idiosyncratic acute liver injury has been developed with promising results. This "in vitro" testing awaits external validation and involves a several week process reducing its potential utility in the clinic [127]. Recently, an updated CIOMS/RUCAM scale, which incorporates an expanded list of alternative causes to be excluded and a new definition of re-challenge, have been proposed but its claimed improved performance needs to be tested in large cohorts of well-characterized DILI cases[128]. A collaborative international working group led by DILIN has been set up to develop an objective, online computer program with a simplified scoring system, evidence-based criteria and refine weighting for wider applicability in the clinical setting.

It is worth noting that the CIOMS/RUCAM scale was developed in the early nineties of the past century. Therefore, this tool did not foresee the particular characteristics of new pharmacological agents that have pointed out to new DILI mechanisms and may present with a prolonged time to onset after drug withdrawal[129].

[H2] ***New biomarkers***

The shortcomings of the traditional DILI biomarkers in terms of liver specificity, prediction of DILI outcome, and mechanistic insight has led to international collaborative efforts to identify and validate new biomarkers[130] (Figure 4).

Both MicroRNA-122 (miR-122) and glutamate dehydrogenase (GLDH) have recently been supported by the FDA for further exploration as liver-specific biomarkers in the clinic[131,132]. miR-122 makes up ~70% of the miRNA content in the liver[133]. Although more liver specific than ALT or AST, substantial inter- and intra-subject variability has been reported in circulating levels in healthy adults[134], which might be due to the release of miR122 from healthy liver cells, which can influence physiology in remote tissues[135,136]; however, the relevance of this variation to use miRNA as a DILI biomarker is not clear since relevant studies have not used similar methods[137].

GLDH is a mitochondrial protein [138] that is not elevated in patients with muscle diseases such as Muscular Dystrophy (Paul Watkins, personal communication). In a large study of healthy volunteers[134], GLDH had a lower inter- and intra- subject variation that miR122. Macrophage Colony Stimulator Factor Receptor (MCSFR) is the receptor on macrophages/monocytes for Colony Stimulating Factor (CSF), a cytokine that controls the proliferation, differentiation, and

function of macrophages. It measurement in blood may reflect activation of innate immune cells (i.e. inflammation). High Mobility Group Protein B1 (HMGB1) a nuclear protein that is released during necrosis of most cell types and can act as a damage associated molecular pattern (DAMP) to activates innate immune cells.

In a recent international collaboration, biomarkers were quantified in serum samples collected from DILI patients within two weeks of DILI onset [134]. While the International Normalized Ratio (INR- a measure of the ability of blood to clot) was the best single biomarker to predict which DILI patients would progress to liver failure, the study showed that osteopontin (OPN) had the best performance of the candidate biomarkers in predicting liver failure, exceeding the traditional liver safety biomarkers including TBIL. This study also addressed whether adding any of the newer biomarkers would improve Model of End-stage Liver Disease (MELD), a model based on traditional blood biomarkers that is used by surgeons to prioritize patients for liver transplantation. It was found that incorporating the values of total keratin 18 (K18) and macrophage colony stimulating factor receptor (MCSFR)[134] resulted in improved prediction of which DILI patients would progress to liver failure. Serum levels of miR122 have also been suggested to predict liver failure outcome from DILI[139], although these findings require further validation. Finally, low blood levels of some cytokines (along with albumin) were reported to be predictive of death within six months of hepatotoxicity onset[140].

[H2] Prognosis

The prognosis of patients with DILI is related to many different factors. Patients detected in population based studies[13,26] have generally more favorable prognosis than patients recruited in tertiary referral centers[11]. In population based cohorts, only approximately 30% of DILI patients present with jaundice whereas this is present in 60-70% of DILI patients seen in tertiary referral centers[11]. The so called “Hy's law” Box 1 named after the late Hyman Zimmerman, is still widely used to predict prognosis in DILI patients[105]. Hy's law was based on the observation that, in patients with isoniazid-induced hepatocellular jaundice[141], the fatality rate from liver failure or the need for liver transplantation was 10% or higher[141]. Afterwards, this ‘10% rule’ has been observed for many drugs and is now used by the FDA to predict the risk of hepatotoxicity of drugs[142,143]. If more than one patient meets the criteria for Hy's law in a clinical trial the implicated drug is unlikely to be marketed as this is likely to lead a hepatotoxicity problem post-marketing [141–143]. The validity of Hy's law has been confirmed in several studies[27,28,107]. Patients with hepatocellular type of jaundice were found to have the worst prognosis in two studies, with a fatality rate of 7-13%[27,28] whereas patients with cholestatic type were found to have highest fatality rate in the first report from the DILIN cohort of 14%[107], which was higher than in the Swedish and the Spanish DILI cohorts with fatality rate of approximately 5-8%[27,28]. However, jaundice induced by different drugs can have different prognosis. For example, in one study of patients with jaundice due to idiosyncratic DILI the mortality rate varied from 40% (6 of 15) for halothane to 0% for erythromycin (0 of 32) [28] Recently, researchers from the Spanish hepatotoxicity network have tried to optimize the definition of Hy's law and to develop a model for predicting ALF in patients with DILI[144]. These researchers were able to develop a prognostic algorithm that was found be more reliable than Hy's law, in particular in predicting who will not develop ALF[144].

Some other biochemical, histological and biochemical features have been shown to affect prognosis. The occurrence of peripheral and hepatic eosinophilia in DILI patients is associated with favourable prognosis in patients with disulfiram induced liver injury[145] and also in many other drugs with well documented hepatotoxicity[98,146]. Although, SJS or TEN rarely accompany DILI, when they do they are associated with a high fatality rate, in particular in those with jaundice[103]. In patients with SJS, mortality is higher in those with severe hepatic dysfunction although it is unclear whether this is due to the effects of the idiosyncratic drug reaction on the liver or if those more severely affected by SJS develop liver dysfunction secondary to sepsis.

The majority of patients with DILI recover completely, and only a small minority experience chronic DILI, defined as persisting liver biochemical or imaging abnormalities at one year and beyond. Only 8% of 292 patients in a prospective Spanish DILI registry developed chronic DILI including liver cirrhosis and ductal lesions with no particular predisposition to any pattern of DILI[147]. Old age, dyslipidaemia and severity of acute episode were risk factors for chronic DILI. Anti-infective and statins were implicated drugs in one-half of patients[147]. In another large cohort study, 9.8% of 1089 patients with DILI died within 2 years; of those where DILI was the primary cause of death, 74% had acute, 13% chronic, 7% acute on chronic, and 6% acute cholestatic failure[148].

[H2] ***DILI detection in Clinical Trials and Post-marketing***

DILI is one of the major reasons for late stage attrition in drug development[1,149,150], and non-negligible safety risks during clinical trials. Careful patient selection, thorough monitoring of clinical symptoms and standard liver chemistries, defined rules for stopping drug administration, as well as systematic signal detection and assessment remain the core elements of DILI risk management.

The FDA "Guidance for Industry, Drug-Induced Liver Injury: Premarketing Clinical Evaluation"[151] has laid the foundation for systematic and standardized diagnosis, assessment, and management of DILI in clinical studies. To minimize risks at early development stages, in line with the FDA DILI guidance, healthy subjects and patients are usually included only if no liver chemistry abnormalities are present at baseline. However, for later stage development trials, once initial assessment of a drug candidate's safety profile is considered satisfactory, inclusion of patients with mild underlying liver abnormalities is encouraged by the FDA to better reflect real-life conditions expected after marketing of the drug.

In the absence of more advanced, fully qualified, sensitive and specific biomarkers, monitoring of liver safety relies on the standard battery of liver chemistry tests: ALT, AST, alkaline phosphatase, and bilirubin (Box 1)[152,153]. Monitoring intervals are adapted to development stage, and preferably to number of patients exposed to the drug and liver safety profile observed before. Typically, liver chemistry is measured twice weekly during phase 1, with frequency decreasing down to once per month during later stage trials, provided no liver safety signal has been observed before[154].

If in clinical trial liver chemistry abnormalities suggest DILI, treatment interruption is the most important measure to avoid progression to more serious injury[151,155]. The FDA DILI guidance offers a set of rules to stop administration of a drug candidate suspected to have caused acute liver injury[151], the first of which recommends discontinuing the drug if ALT or AST exceed 8 x ULN on treatment. However, in development practice, drug administration is mostly stopped at lower levels of aminotransferase elevation to minimize any risk[67,156]. While this conservative approach is taken in the presumed interest of patient safety, premature treatment stop diminishes the opportunity to see adaptation to effects on the liver, if any, in a significant fraction of patients treated[1,157]. Provided close patient observation is ensured, untimely discontinuation of drug administration should rather be avoided to minimize signals falsely suggesting serious toxicity [1,151,157].

As for signal assessment, complementary to standard statistical analysis a systematic workflow using data visualization, based and expanding upon FDA's "eDISH" process[158,159], an interactive visual approach to assessment of hepatotoxicity potential, has been suggested to optimize use of data available and to support proper interpretation of a drug's liver safety profile[160].

For drugs having received regulatory approval despite a pre-marketing signal for potential liver toxicity, regulators will mandate the inclusion of respective safety information and risk mitigation measures in the product label. Depending on the severity of the signal, this may be mentioning of hepatotoxicity in the Adverse Reactions section, in the Warnings and Precautions section, or even in a dedicated Boxed Warning section, along with stipulation of monitoring intervals for liver chemistry tests. A key problem in the post-marketing setting though is that monitoring intervals specified in the label are not always strictly followed, potentially increasing the risk of liver toxicity[161–163].

If liver safety of a new drug candidate cannot be fully established in pre-marketing trials, further studies may be required after regulatory approval of the drug to assess potential hepatotoxicity (Box 2).

[H1] Management

In many patients, DILI can spontaneously improve without the need for active treatment. The key steps in the management of DILI are the timely recognition and withdrawal of the offending medication(s), the timely referral of individuals with drug induced ALF to a liver transplant center, and pharmacotherapy (Figure 3). Delay in timely identification and immediate withdrawal of isoniazid and other anti-tuberculosis medications is considered as one of the risk factors for worse outcomes such as liver transplantation or death[7]. Rechallenging with a suspected agent is strongly discouraged unless clinically imperative and in such instances starting at a lower dose and frequent biochemical monitoring is advised[164]

[H2] Pharmacological therapy

Therapeutic options for hepatocellular DILI are limited. Corticosteroids are frequently administered in patients with significant DILI (e.g., associated with liver dysfunction) in an

empiric fashion, but there is no evidence to support their use except in instances where acute autoimmune hepatitis cannot be excluded or to treat hepatotoxicity due to immune checkpoint inhibitors (ICIs). Currently, the mainstay for treating hepatotoxicity due to ICIs is prednisone, with additional or alternate immunosuppressant such as mycophenolate mofetil[165] although the evidence to support this is inconclusive at best. In a recent experience[166] with 100 patients who had at least Grade 3 hepatotoxicity according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 (ALT ≥ 5 x ULN) from ICIs. Corticosteroids were administered in 67 patients, with all but three responding to steroid therapy. However, the decision to start corticosteroid therapy in this population remains controversial. In a recent study the management of patients with ICI-induced liver injury was tailored according not only to biochemical (bilirubin >2.5 mg/dL and/or international normalized ratio, INR >1.5) but also histological markers of severity. Using these pre-established guidelines 6 out of 16 (38%) patients with ICIs liver damage did not receive corticosteroids and spontaneously improved[167]. In another cohort of 128 melanoma patients treated with ICIs, only half of the patients with DILI (5/10) received steroids and resolution of DILI occurred in all patients with a median time of 4.7 weeks in those receiving no steroids compared to 8.6 weeks in those who received corticosteroids[168]. A suggested algorithm to detect and manage hepatotoxicity due to ICIs in patients with cancer who are considered for ICI therapy in accordance with current practice is shown in FIG. 5.

Cholestyramine, a bile acid resin, can be administered to patients with acute liver injury due to leflunomide, an immunomodulatory agent used in the therapy of rheumatic arthritis and psoriatic arthritis to accelerate elimination of this drug [7]. *N*-acetylcysteine (NAC), an antidote for acetaminophen toxicity, was investigated in a randomized placebo controlled trial for non-acetaminophen ALF that included DILI as one of the subgroups[169]. The transplant-free survival of individuals with non- acetaminophen ALF due to DILI who received NAC was 58% and was significantly higher than those who did not receive NAC (27%, $P<0.05$). In individuals with cholestatic DILI with significant itching may benefit from anti-histamines such as diphenhydramine and hydroxyzine or bile acid resins such as cholestyramine. It is not uncommon for clinicians to try ursodeoxycholic acid (UDCA) in individuals with significant cholestatic DILI; in fact, as many as 30% of patients in the DILIN prospective study were given UDCA[164]. But, there are no data to support its use in DILI.

[H2] Liver transplantation

While there are no strict criteria in terms of when to initiate a referral for liver transplantation, a general rule of thumb is the development of acute liver failure as evidenced by coagulopathy, early mental status changes, or renal dysfunction. In individuals with hepatocellular DILI, progressive worsening of jaundice should also prompt the clinicians to consider initiating a referral to a near-by liver transplant center.

[H1] Quality of life

After experiencing a severe adverse drug reaction (ADR), many patients develop fear and anxiety toward medications[170] i.e., possible recurrence, re-exposure to the drug, impact on their fertility, or developing ADRs due to other drugs. Such a negative perception of medications can adversely

affect their quality of life (QOL) and patient's adherence to the treatment and may increase the discontinuation of needed therapy[171]. The most widely accepted questionnaire to measure QOL is the SF-36[172], a standardized tool used to assess patient health across eight dimensions. An alternative method is the Beliefs about Medicine Questionnaire (BMQ)[173].

As observed in cutaneous ADRs[170], patients who experienced DILI could develop fear, anxiety, disbelief toward medicines, and discomfort, all of which can deteriorate their QOL. However, in idiosyncratic DILI the analysis of the impact of the disease in terms of the QOL of patients remains a neglected area of research. In a study conducted in South Korea in patients suffering from a DILI episode, the authors found greater indexes of anxiety and depression in patients with liver injury induced by herb and dietary supplements compared with healthy population and patients with liver disease from other etiologies[174]. Interestingly, the DILIN group documented that patients with persistent liver enzyme elevation 12 months after DILI onset had significantly poorer SF-36 physical summary scores at DILI onset and throughout follow-up compared to those who resolved[175].

Acetaminophen overdose is the most common cause of drug-induced ALF in the US[176]. Whereas only 25% of idiosyncratic drug-induced ALF achieve spontaneous recovery, that rate is over 65% in patients with acetaminophen-induced ALF[176]. Despite the better short-term orthotopic liver transplantation (OLT)-free survival in acetaminophen-induced ALF, spontaneous survivors (i.e., recovery without OLT) from acetaminophen-induced ALF report lower general health scores, a longer duration of impaired mental and physical health, and a longer duration of activity limitations due to poor health, pain, depression, and anxiety compared to non-acetaminophen ALF spontaneous survivors and OLT patients (of different etiologies including idiosyncratic DILI)[177]. This apparent contradiction, however, could be explained by the fact that acetaminophen survivors had significantly higher rates of psychiatric and substance abuse disorders[177].

Taken together, although the evidence is limited, patients appear to have poor physical and psychological status and low QOL after certain types of DILI presentation, such as persistent liver enzyme elevation and ALF with and without OLT. Otherwise, we lack studies of QOL in the wide spectrum of DILI phenotypes as well as studies assessing the beliefs, attitudes, and expectations after an episode of hepatotoxicity for both patients and physicians. Interestingly, a survey performed in 2014 found that primary care physicians shared several liver safety concerns regarding prescriptions of statins despite its safety and efficacy, leading to their underutilization[178].

An integrative holistic model that takes into account not only the liver sequels imposed by DILI but also its overall impact on patient's health should encourage the evaluation of QOL. Hence, it would be essential to conduct a QOL survey with each patient during and after the acute phase of the DILI episode.

[H1] Outlook

Prediction of DILI risk with preclinical cell and organelle based assays and chemical properties of drugs promises to enable selection of the most favorable characteristics among a group of compounds to advance to in vivo testing in drug development. Several issues will need to be further investigated in the future, such as how the identification of drug induced hazards, such as oxidative stress, ER stress, among others, inform on the pathogenesis of idiosyncratic DILI, and if these stressors are necessary for idiosyncratic DILI which is largely immune mediated or if they surrogates for hepatic exposure to and metabolism of lipophilic drugs. In addition, whether the fitness of adaptive responses to these stressors (e.g. UPR^{ER}, UPR^{mito}, mitochondrial quality control, antioxidant defense, induction of alternative routes of transport or detoxification of bile acids or drug metabolites, dampens the progression from minimal to severe liver injury remains to be established. Furthermore, elucidation of the role of immune tolerance as a mechanism of adaptation to dampen progression may potentially lead to novel approaches to prevent severe injury. Recent attempts to integrate mechanisms and patient risk factors using quantitative systems toxicology modelling are showing promise towards predicting DILI risk[179].

Another important area for research is the suppression of cholestasis and cell death, as well as innate (sterile) immune responses, as an approach to treating established acute liver injury. Therefore, the role of various cell death pathways and cholestatic injury mechanisms need to be identified in intrinsic and idiosyncratic DILI to exploit new therapies to suppress overt liver injury as it reaches certain thresholds which predict advancement of injury, perhaps informed by early identification of predictive biomarkers.

It is unrealistic to expect medications to be entirely free from adverse effects; therefore, discovery and development of diagnostic, prognostic and mechanistic biomarkers enhancing safe use of medications are integral to precision medicine. Beyond the emerging biomarkers already discussed earlier in this article, cutting-edge technologies such as the mass cytometry, single cell genetics and next generation sequencing would permit in-depth immunophenotyping of circulating and infiltrating immune cells as well as microRNA profiling potentially identifying patterns unique to DILI. With discovery science informed by recent advances in the understanding of the pathogenesis, use of advanced analytical methods and tools such as deep machine learning would bring about a step change in the application of a combination of biomarkers in an individual clinical scenario to support decision-making.

Accurate and confident diagnosis is the most important step in the management of DILI as prompt withdrawal of the causative agent is the only intervention necessary in the majority of cases; clear diagnosis also prevents re-exposure with its serious consequences. Genome-wide association studies led by international consortia have identified a number of genetic risk factors for DILI; HLA genotypes and haplotypes have been associated with hepatic adverse reactions related to over 20 drugs. HLA genotyping is widely accessible, affordable and can assist diagnosis in selected clinical scenarios[180]. High negative predictive values (>95%) of these alleles can be used to rule out particular drug as an causative agent when pre-test probability of DILI is low and an alternative competing diagnosis exists. Carriage of a specific HLA allele favours attribution of liver injury to a particular drug when exposure to a combination of drugs does not permit definite conclusions. HLA typing could be an adjunct in the differential diagnosis of DILI versus autoimmune hepatitis (AIH), as with International AIH diagnostic criteria which attributes

additional scores for carriage of HLA-DRB1*0301 and DRB1*0401[181]. Performance characteristics of HLA alleles used as a test in DILI cases are comparable to autoantibodies and immunoglobulin profile that are performed routinely in the investigation of acute liver injury[96] .

There is a significant overlap among HLA alleles associated with a variety of adverse reactions including DILI, cutaneous hypersensitivity and drug-induced pancreatitis. Hence, one potential consideration is to treat all relevant HLA genotypes as one panel covering different forms of adverse drug reactions, thereby improving its clinical application[180]. More recently, GWAS have revealed non-HLA genetic variants associated with DILI secondary to Interferon-B[182] as well as a DILI in general[183]. In addition, it has been estimated that approximately 30–40% of functional variability in pharmacogenes can be attributed to rare variants requiring sequencing based approaches for discovery[184].

As with most polygenic disorders genetic tests have not been used so far to risk stratify individuals prior to prescription with an intention to prevent DILI. With an aim of introducing polygenic risk prediction into clinical care investigators recently developed and validated genome-wide polygenic scores for 5 common diseases[185]. Truly individualized medicine would be realized when a similar polygenic score related adverse drug reactions is developed ready for clinical application.

From a therapeutic standpoint idiosyncratic DILI is still an orphan disease. This is the consequence of a number of factors. First, the incomplete understanding of the DILI pathogenesis and the complexity of its underlying mechanisms have hampered the efforts to develop animal models relevant to human idiosyncratic DILI. Despite the efforts to establish a better approach to human DILI, such as inhibiting normally tolerogenic immune pathways to render mice susceptible to DILI[186], there is no widely accepted animal model and none of the *in vitro* and *in silico* existing models of hepatotoxicity are approved by the regulatory agencies for preclinical drug development. On the other hand, the discovery of mechanistic biomarkers along with genetic information brings hope for improving the detection of DILI in clinical trials. The current absence of diagnostic DILI biomarkers impairs an accurate DILI case qualification process, which is crucial to correctly enrol patients in trials to assess older or new molecules in the treatment of this condition. Presumably, international efforts already in place (Translational Safety Biomarker Pipeline, TransBioLine) to further discover and validate specific DILI biomarkers will change the landscape over the next years. Last but not least, the relative rarity of the disease along the myriad of phenotypic presentations, which further reduces the potential randomization of eligible cases, precludes the undertaking of statistically powered clinical trials. Nevertheless, evaluation of the potential benefit of older agents empirically used in DILI, such as UDCA and steroids, is worthy of well-designed clinical trials. This is nowadays feasible taking advantage of international consortia that prospectively recruit bona fide DILI cases. In fact, prospective DILI registries will remain as an invaluable resource for testing diagnostic biomarkers and promoting new therapeutic strategies in the near future.

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Box 1. DILI criteria.

[H1] Clinical chemistry criteria

An international expert panel recommended DILI to be considered when any one of the following thresholds are met even in the absence of symptoms:

- Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) elevation ≥ 5× ULN (upper limit of normal)
- Alkaline phosphatase (ALP) elevation ≥ 2× ULN or
- Total bilirubin (TBIL) concentration exceeding 2× ULN associated with ALT/AST elevation ≥ 3x ULN[104].

[H1] Detection in clinical trials, Hy's law

Key signals for potential DILI are imbalances in aminotransferase elevations across treatment vs control groups, and, as an indicator for more serious injury, the combination of aminotransferase and bilirubin elevations matching so-called Hy's law criteria (which identifies individuals with hepatocellular jaundice), consisting of three components:

- 3-fold or greater aminotransferase elevations above ULN more frequent as compared to (nonhepatotoxic) control or placebo,
- Subjects showing ALT or AST > 3 x ULN, combined with elevation of serum TBIL to > 2 x ULN, without initial findings of cholestasis, indicated by elevated ALP,
- Absence of any alternative cause likely explaining the liver test abnormalities[151].

Hy's law is a reasonably sensitive and specific predictor of a drug's potential to cause serious hepatotoxicity[142], indicating hepatocellular injury severe enough to impair hepatic function[151,187], and it is the FDA's key marker to screen for a drug candidate's liver toxicity risk[143].

Box 2 Postmarketing Pharmacovigilance.

As drug-induced liver injury (DILI), in particular idiosyncratic forms, is a rare yet serious Adverse Drug reaction, the likelihood to detect a robust signal before marketing authorization, even given increasingly large trials in drug development programs, is low. Thus, in the absence of clear-cut Hy's law cases, there is a genuine risk that a signal is observed only after launch of the product[151,188], either during post-marketing surveillance (PMS) studies, specific DILI registries, or from spontaneous reporting. While dedicated PMS studies and registries help to generate high quality data and structured output, unsolicited spontaneous reports often lack adequate quality and completeness to support timely detection and causality assessment of suspected DILI post marketing. Key challenges comprise, on top of a wide-spread lack of awareness for DILI in clinical practice,

- Missing baseline liver chemistry values
- Lack of adherence to recommended monitoring intervals, even with products that carry a boxed warning for DILI[161–163]
- Treatment with multiple drugs, including self-medication e.g. with herbals and dietary supplements.

To address these challenges and overcome respective deficiencies, more in-depth training on background, detection, and management of DILI for physicians in hospital and clinical practice may be helpful.

Figure 1: Hepatocyte transporters and cellular mechanisms of drug-induced liver injury

Blood plasma enters the perisinusoidal Space of Disse through the fenestrated liver sinusoidal endothelium and is in direct contact with the basolateral surface of hepatocytes. Drugs are taken up from sinusoidal blood by various transporters located in the basolateral hepatocyte membrane, including members of the organic anion transporting polypeptide (OATP), organic anion transporter (OAT) and organic cation transporter (OCT) families. Examples of specific drugs uptaken by each transport are provided in orange boxes. The process of drug metabolism and elimination in hepatocytes occurs in three phrases. The metabolism of drugs by cytochrome P450 (CYP) enzymes can generate reactive oxidative metabolites which are potentially toxic to the cell (phase 1) through covalently binding to cellular proteins, thereby inhibiting the function of the protein, or by causing cell stress. Drug metabolites are conjugated to endogenous molecules (phase 2), following which, they are eliminated from the cell via the bile salt export pump (BSEP) or multidrug resistance gene product (MDR). Cell injury releases protein adducts that can act as neoantigens, triggering an immune response in susceptible individuals. Drug metabolites can also inhibit the function of the hepatocyte canalicular efflux transporters such as BSEP, causing an increase in intracellular bile acid concentrations that damage mitochondria and lead to apoptosis. Bile acids induced stress may also lead to increased targeting of death receptors to the plasma membrane and sensitize to ligand (TNF, FasL) induced apoptosis or necrosis or induce ligand independent activation of death receptors. DILI is frequently caused by a combination of intrinsic mechanisms such as inhibition of BSEP and toxicity to mitochondria, with subsequent immune damage to hepatocytes. OST, organic solute transporter; MRP, multidrug resistance associated protein; MATE, multidrug and toxin extrusion transporter; APC, antigen presenting cell.

Figure 2: Molecular Mechanisms of idiosyncratic and intrinsic DILI.

DILI is most often caused by lipophilic drugs which are converted to reactive metabolites which have the potential to covalently bind to proteins leading to cellular organelle stress. The reactive metabolite may target proteins of mitochondrial or ER and induce mitochondrial or ER stress, which promotes organelle specific adaptive responses to increase chaperone proteins which protect against misfolding in organelles or antioxidant response through gene regulatory programs triggered by redox activated transcription factors (Nrf2). When the adaptive responses are inadequate, the liver cell progresses to lethal consequences mediated either by collapse of the mitochondrial function (MTPT) and necrosis or to activation of regulated cell death pathways involving permeabilization of the outer mitochondrial membrane (MOMP) due to activation of pore forming proteins such as Bax, Bak, Bid leading to release of cytochrome c, caspase activation, and apoptosis. Alternatively, in theory, other programmed cell necrosis mechanisms may contribute, such as necroptosis (RIPK1/3/MLKL) or pyroptosis (caspase1 or 10 cleavage of gasdermins) which permeabilize the cell membrane, or ferroptosis (iron dependent lipid peroxidation) may contribute to DILI but remains unproven. Lethal or sublethal organelle stress may release DAMPs such as HMGB1 or DNA, which activate TLR leading to proinflammatory cytokine/chemokine release. The inflammatory response amplifying cell death in intrinsic DILI, depending on the acuity and severity of injury or may actually promote resolution. In contrast, innate immune response may provide danger signals to amplify adaptive immunity in idiosyncratic DILI. The key is that HLA polymorphisms which favor presentation of drug adducted

peptides can be HLA restricted so that mainly individuals carrying the HLA variant are susceptible to developing an adaptive immune response which typically leads to a T cell response directed at hepatocytes; usually cytotoxic CD8 T-cells which target the peptide-drug exposed on MHC1 class molecules the hepatocytes, though sometimes leading to antibody dependent cytotoxicity. It is proposed that the majority of patients who have a genetic HLA predisposition do not experience significant injury because most develop immune tolerance. Therefore, it is speculated that progression to overt IDILI may be due to impaired immune tolerance.

Figure 3: **A suggested algorithm to suspect, diagnose, and manage idiosyncratic DILI.**
Drug induced liver injury should be suspected in any individual presenting with acute liver injury, unexplained chronic hepatitis, or unexplained worsening of chronic liver disease, or acute on chronic liver failure. In such instances, careful history of prescription, over-the-counter, and complementary and alternate medications should be taken. In general, it is a good practice to hold the suspected agent(s) while the work-up for competing etiologies is undertaken. The work up for competing etiologies should be tailored according to the clinical presentation, but generally consists of testing for acute viral hepatitis, hepatobiliary imaging, and autoimmune serologies. If the competing etiologies are excluded, one should permanently discontinue the offending agent unless it is very important for clinical management. In cases of DILI where there is evidence for acute liver failure, a prompt referral to a liver transplant center should be considered. As some patients with DILI may develop chronicity, it is important to follow-up patients for next 12 months to ascertain normalization of liver biochemistries and liver function.

Abbreviations: DILI: Drug induced liver injury; CLD: Chronic liver disease; ACLF: Acute on chronic liver failure; CAM: Complementary and alternate medicines

Figure 4: Traditional and Investigational Biomarkers of DILI.
An active area of research is the identification of biomarkers that could detect initiation of each of the pathophysiological steps of DILI. During hepatocyte necrosis, there is release of miR122, glutamate dehydrogenase (GLDH), and full-length cytokeratin 18 (Keratin 18-FL). It has been proposed that by processing fresh blood to remove intact mitochondria, GLDH can identify mitochondrial toxicity as a mechanism of DILI[189]. The serum ratio of caspase-cleaved cytokeratin 18 to full length cytokeratin 18 (cc18/k18) has been proposed to estimate the ratio of apoptosis to necrosis during DILI. HMGB1, mir122 and DNA are among multiple damage-associated molecular patterns (DAMPS) that activate innate immune cells, which in turn release MCSFR1. OPN is involved with migration and infiltration of inflammatory cells and also appears to promote regeneration. Identifying biomarkers of innate immune cell activation in the liver is ongoing. Acetylated HMGB1 was proposed to address this but the integrity of at least one of the key studies has been questioned (https://www.ncbi.nlm.nih.gov/pubmed/29729369). Abbreviations: HMGB1: High mobility group box 1 protein; ALT: Alanine aminotransferase, AST: Aspartate aminotransferase; INR: International Normalized Ratio; TBL: Total bilirubin; DAMPs: Damage-associated molecular patterns; MCSFR1: Macrophage colony-stimulating factor receptor 1; OPN: Osteopontin; HSCs: Hepatic stellate cells

Figure 5: **A suggested algorithm to detect and manage hepatotoxicity due to immune checkpoint inhibitors in patients with cancer.**

In patients with malignancies who are considered for immune checkpoint inhibitor (ICI) therapy, baseline evaluation consisting of liver biochemistries and liver function tests, viral hepatitis serologies, and autoimmune markers should be undertaken. If there is underlying liver dysfunction, ICI therapy may not be suitable unless the underlying liver dysfunction is suspected due to malignancy. In patients without serious underlying liver disease, ICI therapy may be initiated but with serial liver biochemistry monitoring every 1-3 weeks, depending on local practice. If there is emergence of elevated liver biochemistries, they should be managed according to their levels. For patients with ALT levels > 1 but < 3 ULN or total bilirubin elevation up to 1.5 mg/dl, one may cautiously continue the ICIs but should consider accelerated liver biochemistry monitoring. For patients with incident ALT levels > 3 to ≤ 5 ULN or total bilirubin 1.5-3 mg/dl, one should consider temporarily discontinue the ICIs while initiating a work up for competing etiologies and also consider initiate therapy with prednisone at 0.5-1 mg/kg dose. If there is no rapid response, one may have to add additional immunosuppressive therapy with mycophenolate or increase the prednisone dose. For patients who develop ALT > 5 ULN or total bilirubin > 3 mg/dl, ICIs should be permanently discontinued and they should be initiated therapy with 1-2mg/kg prednisone.

Abbreviations: ICI: Immune Checkpoint Inhibitors; ALT: Alanine aminotransferase; T Bili: Total bilirubin; Hep B S Ag: Hepatitis B Surface Antigen; Hep B Core Ab: Hepatitis B Core Antibody; Hep C Ab: Hepatitis C antibody; Hep C PCR: Hepatitis C polymerase chain reaction; ANA: Antinuclear antibody; ASMA: Anti smooth muscle antibody; ULN: Upper limit of normal

Table 1: A working theory: drug-host interplay in drug-induced liver injury

Drug factors	Host factors	Effect on DILI risk
Drug exposure to hepatocytes		
High daily-recommended dose, longer administration	Bioavailability, transporters, drug metabolizing enzymes	Increased drug exposure to hepatocytes increases the likelihood of inducing drug's toxic effects.
Toxicological effects on cellular homeostasis		
High potency of drug toxicity	Cellular senescence, impaired cellular adaptation	Drug's toxic effect exceeding the host's coping mechanisms leads to an increased likelihood of cellular dysfunction/death.
Reactive metabolite formation	Increased drug metabolizing enzyme activities	Increase reactive metabolite formation
	Lysosomal dysfunctions	Impaired functions to maintain cellular homeostasis
Mitochondrial toxicity	Mitochondrial dysfunction, older age,	Enhance mitochondrial damage
	Impaired mitophagy	Impaired functions to maintain mitochondrial homeostasis
Oxidative stress induction	Reduced anti-oxidants	Increased cellular damage due to oxidative stress
	Female, estrogens (increased anti-oxidants)	Protective against cellular oxidative stress
BSEP inhibition	Older age (reduced ATP supply), reduced activities of other bile acid transporters (e.g., MRP2,3,4)	Enhancing bile acid accumulation in the hepatocytes leads to cellular damage.
Immune response, inflammation, and tissue injury		
Immunomodulatory drugs	HLA genotypes, immune senescence, sex hormones	Intensified or dysregulated immune response augments inflammation and tissue injury.
Tissue repair		
Drugs impairing tissue repair	Older age, cirrhosis	Impaired tissue repair augments tissue damage, leading to a serious outcome.

Table 2. Case definitions and phenotypes of drug-induced liver diseases

Most patients with acute drug-induced liver injury (DILI) in clinical practice are characterized based on their liver biochemistry, as hepatocellular, cholestatic or mixed pattern of DILI. As the pattern of elevated liver enzymes may evolve over the course of the event[112], categorization of DILI is based on the first set of laboratory tests available in relation to the clinical event[104]. Ratio (R value) of alanine aminotransferase (ALT) (or aspartate aminotransferase, AST) activity expressed as fold elevation over its upper limit of normal laboratory range to alkaline phosphatase (ALP) activity is used to define patterns of DILI. The pattern of liver injury has implications for prioritizing immediate investigations essential to exclude alternative causes of the event as well as outcome. Hepatocellular cases are more likely to resolve rapidly, but are associated with higher hazard ratio for fatality[144,148]. Other patterns of DILI should be characterized according to imaging/ histological findings.

	Case definition	**Drugs associated with phenotypes**
Hepatocellular pattern of DILI		
	ALT (or AST) alone is elevated ≥ 5 fold above ULN or R ≥ 5	Acetaminophen, isoniazid, rifampicin, pyrazinamide, diclofenac, lamotrigine, minocycline, nitrofurantoin, nevirapine, efavirenz, sulfonamide, disufiram, fenofibrate
Cholestatic pattern of DILI		
	ALP alone is elevated ≥ 2 fold above ULN or R ≤2	Chlorpromazine, erythromycin, penicillins, amoxicillin-clavulanate, flucloxacillin, cephalosporins, sulfonamide, terbinafine, androgens, oral contraceptives

Mixed pattern of DILI		
	R >2 to <5	Phenytoin, carbamazepine, lamotrigine, sulfonamides
Autoimmune-like hepatitis		
	Presenting features of acute or chronic DILI with serological and/or histological markers of idiopathic autoimmune hepatitis	Nitrofurantoin, α-methyl-dopa, minocycline, diclofenac, statins, adalimumab, infliximab, herbals
Liver injury related to immune check points inhibitors (ICIs)		
	Acute hepatitis, may be severe. Histological pattern include granulomas and central endothelitis (anti-CTLA-4) or lobular hepatitis(anti-PD-1/anti PDL-1)	Ipilimumab (anti-CTLA-4) Nivolumab, darvolumab, pembrolizumab (anti-PD-1/anti PDL-1)
Drug reaction with eosinophilia and systemic symptoms (DRESS)		
	Drug-induced hypersensitivity reaction involving skin and internal organ involvement	Carbamazepine, phenytoin, phenobarbitone, allopurinol, lamotrigine, dapsone, sulfonamide, nevirapine
Drug associated fatty liver disease (DAFLD)		
	Non-alcoholic fatty liver disease attributable to exposure specific medications	Amiodarone, methotrexate, tamoxifen, 5-fluorouracil, irinotecan
Acute fatty liver (microvesicular steatosis)		

	Rapid liver involvement with extensive microvesicular steatosis	Amiodorone, didanosine, stavudine
Nodular regenerative hyperplasia (NRH)		
	Diffuse nodularity within the liver with wide and narrow sheets of hepatocytes at the centre and periphery of nodule respectively without advanced fibrosis leading to noncirrhotic portal hypertension	Azathioprine, 6-thioguanine, oxaliplatin, busulfan, bleomycin
Vanishing bile duct (ductopenic) syndrome		
	Cholestasis associated with gradual loss of intrahepatic bile ducts.	Azathioprine, amoxicillin-clavulanate, carbamazepine, chlorpromazine, erythromycin, flucloxacillin, phenytoin, terbinafine and co-trimoxazole.
Secondary sclerosing cholangitis		
	Acute DILI with histological and/or MRCP features similar to those of primary sclerosing cholangitis.	Amiodarone, atorvastatin, amoxicillin-clavulanate, infliximab, 6-mercaptopurine, and venlafaxin
Peliosis hepatis		
	Characterized by randomly distributed blood-filled cavities	Anabolic steroids, oral contraceptives, vitamin A

Hepatocellular adenoma, carcinoma		
	Characteristics of hepatocellular adenoma or carcinoma based on imaging studies or histology	Contraceptive steroids, danazol, androgens

Abbreviations: MRCP: magnetic resonance cholangiopancreatography

Table 3. Laboratory, imaging and histological assessment in DILI diagnosis.

Assessment	Diagnostic value
Elevated aminotransferases (ALT, AST)	Hepatocellular damage, not liver specific, towering values suggest hypoxic damage of the liver
Elevated creatine kinase (CK)	In association with elevated AST/ALT indicates muscle injury rather than liver damage
Elevated total bilirubin (TBL)	Impaired hepatic uptake, conjugation or excretion, biliary obstruction, haemolysis. Isolated elevation even of the conjugated fraction does not mean DILI. Of diagnostic and prognostic value when associated to a rise in ALT (Hy's law)
High alkaline phosphatase (ALP)	Cholestasis if bone disease can be excluded, also elevated in biliary obstruction and infiltrative diseases
Elevated gamma-glutamyl transferase (GGT)	Indicate cholestasis when associated to a rise in ALP, isolated elevation is not indicative of liver injury. Concomitant elevation of mean corpuscular volume suggests alcoholic liver disease
Low albumin, high INR	Impaired hepatocellular function, altered in cirrhosis of any cause
Serology hepatitis A, B, C, E	Viral hepatitis. IgM anti-HAV, IgM antiHBc, HBs Ag, HBV DNA if HBsAg carrier; HCV RNA and IgM & IgG anti-HEV, HEV RNA
Serology for CMV, HSV, EBV infection	Always in cases with systemic symptoms; IgM & IgG anti-CMV, IgM & IgG anti-HSV; IgM & IgG anti-EBV
ANA & ASMA, IgG	Autoimmune hepatitis (may be drug-induced)
Ceruloplasmin & transferring saturation & Alpha-1-antitrypsin	Wilson disease & Hemochromatosis (in anicteric hepatocellular damage) & Alpha-1-antitrypsin deficiency, respectively
Imaging -Ultrasonography	Normal in DILI, mandatory to exclude focal lesions and biliary tract disease. No additional imaging techniques required in "viral hepatitis like" syndrome
-MRI	Necessary in cholestasis and/or accompanying abdominal pain; Biliary tract disease (benign/malignant) may require endoscopic retrograde cholangiopancreatography in addition to MRI. Also help to exclude Non-alcoholic fatty liver disease; focal lesions; ischemic injury
Liver biopsy	Autoimmune hepatitis phenotype; liver injury related to immune check point inhibitors; suspected atypical DILI presentations (i.e. sinusoidal obstruction syndrome, peliosis hepatis, microvesicular steatosis); negative or incomplete dechallenge (for assessing severity and/or competing etiologies)

Abbreviations: ANA, anti-nuclear antibody; ASMA, anti-smooth muscle antibody; GGT, gamma-glutamyl transferase; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; Ig G, immunoglobulin G; MVS, microvesicular steatosis

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